GENITOURINARY MEDICINE

Editorial

The role of HIV-proteinase inhibitors

Two recent advances have engendered substantial optimism amongst both physicians and patients that the treatment for HIV infected individuals is improving. Firstly, three large clinical endpoint studies have demonstrated that using combinations of two nucleoside analogues (zidovudine (ZDV) with either didanosine or zalcitabine) significantly improved survival compared with zidovudine alone.[1–3] Secondly, two clinical endpoint studies involving HIV-proteinase (or protease) inhibitors in nucleoside analogue-experienced patients with advanced HIV disease have demonstrated significant survival benefits.[4 5] Three such agents are licensed in the USA and pending licensing in Europe (saquinavir, indinavir and ritonavir) with a number of others in clinical development.

The understanding that the replication of HIV is dependent upon an aspartic viral proteinase which acted upon a unique amino acid bond (phenylalanine to proline) not cleaved by mammalian enzymes,[6] gave promise that this would represent an excellent target for drug development. Crystallisation of the HIV proteinase enabled elucidation of its structure, a symmetric homodimer derived from two identical 99 amino acid residues, and rational computer-based design of inhibitor molecules.[7] These inhibitors show substantial antiviral activity against both HIV-1 and -2 and ZDV-resistant strains *in vitro* with wide therapeutic index.[8 9] Whilst one promising drug proved inactive *in vivo* because of tight binding to alpha acid glycoprotein, this problem does not affect the activity of those drugs currently available. All of these compounds are metabolised in the cytochrome P450 enzyme system, mostly the CYP3A4 isozyme, and particularly in the case of ritonavir, may both induce and competitively inhibit this enzyme. This leads to a number of clinically important drug interactions which are maximal with ritonavir, and less prominent with indinavir and saquinavir.

Excitement was generated by these compounds when initial short-term studies involving small numbers of patients showed substantial improvement in activity markers of HIV infection including CD4 count rises and viral load reductions.[10–12] Both of these markers are established predictors of clinical outcome and recent studies have indicated that changes in them predict at least part of the treatment effect of drugs.[13]

Activity marker effects

Impressive reductions in viral load greater than one $\log_{10}$ have been observed with both ritonavir, indinavir and high doses of saquinavir in 24 week monotherapy studies. When sub-optimal doses of these drugs are given, the viral load response is less impressive with a more rapid return to baseline. Subsequent increases in the dose do not result in further reductions in viral load.[14] This suggests compliance will be a major issue in gaining prolonged therapeutic benefit from these drugs. Lower than recommended doses of both ritonavir and indinavir rapidly select for resistant virus which in some instances is cross-resistant to other proteinase inhibitors. However, resistance may be delayed by consistent dosing at optimal levels and by co-administration with nucleoside analogues. Additionally, delay in ZDV resistance has been reported in combination with saquinavir or indinavir.

Responses in activity markers, particularly the duration of response, are maximal when used with one or two nucleoside analogues. This has been demonstrated over 24 weeks with combinations of indinavir with ZDV and lamivudine,[15] indinavir with ZDV and didanosine (ddI),[16] and either ritonavir or saquinavir with ZDV and zalcitabine (ddC).[17 18]

A significant proportion of individuals given a proteinase inhibitor combined with two nucleoside analogues may achieve reductions in viral load to below the detectable limit of the assay (200 copies per ml). Obviously such data should not be taken to indicate that viral replication has been completely stopped in all body compartments or that virus has been eradicated from the body. Lower levels of viral replication may still be present in the plasma and, more importantly in other sites. Initial experiments indicate that there is a corresponding reduction in viral load in lymph nodes very similar to that seen in plasma but the CNS penetration of these agents has not yet been fully determined.

Such very large reductions in viral load are encouraging and are likely to translate into improved clinical outcome. However, it is surprising that despite these very massive reductions in viral load, the increment in CD4 positive cells is only between 100 and 200/mm^3 rather than a restoration to normal levels. Data from patients with relatively advanced HIV disease indicate that irreversible destruction of the immune system has occurred involving the loss of dendritic cells within the lymph nodes or deletions of specific Vβ families within the CD4 positive subset.[19] Following proteinase treatment the CD4 + count rises predominantly from the CD4 R0 or memory subset with little change in the naive thymus derived CD4 RA cells. This may result in some improved functional immune capacity[20] but disease progression may still occur despite a CD4 or CD8 response.[4]

Clinical studies

Whilst the responses of viral load to treatment with proteinase inhibitors are extremely impressive, it is important that such responses should improve clinical outcome and so

it is heartening that the results of two clinical controlled trials are now available, both of which show improvements in clinical outcome associated with the addition of a proteinase inhibitor to other treatment.

In the first study which has now been published in abstract form, patients with very advanced HIV disease (mean CD4 count at study entry of 32/mm^3) and at least nine months prior nucleoside therapy were shown to have an improvement in the development of new AIDS events or death when given ritonavir compared with placebo treatment.[4] Participants continued whatever nucleoside analogues they were on at study initiation although a significant minority were on no other treatment. Although the short term improvement in both survival and development of new clinical events was impressive, this was only of the same order of magnitude as was originally seen in the initial ZDV placebo controlled study.[21] Although viral load drops in excess of 1 log were seen in the initial weeks following ritonavir therapy, the viral load drop by the end of six months was only on a median of 0·6 logs implying that the improvement in outcome might only persist for a relatively short period.[4]

In the second study saquinavir, or ddC and saquinavir/ddC combinations were compared in patients clinically failing or intolerant to ZDV therapy. Saquinavir/ddC combination produced a significant improvement in mortality and clinical outcome compared with either monotherapy. This study has not yet been reported in detail.[5]

Timing of treatment with proteinase inhibitors

It is likely that the treatment with proteinase inhibitors will be a very important component of antiretroviral therapy. Many people believe that optimum treatment would be maximal antiretroviral therapy given early. This early "hit hard" view would imply giving triple or quadruple combinations including at least two nucleosides and a proteinase at a relatively early stage of disease when the immune system is intact. This would include all individuals with a CD4 count below 500 cells/mm^3 as well as individuals with a CD4 count above this if they had a viral load level associated with poor prognosis.[22] It is not clear if such an approach is necessarily better than starting therapy with two dideoxynucleoside analogues and switching to a different nucleoside combination with the addition of a proteinase following apparent treatment failure as judged by clinical deterioration, changes in viral load or CD4 count. Interestingly a fair proportion of patients prefer this approach as it does hold in reserve one of the most potent classes of agents available for the treatment of HIV. Which is the better strategic policy will obviously have to await further research.

It is also unclear which of the three proteinase inhibitors should be used initially. All three have very similar activity *in vitro*[23] but the present formulation of saquinavir is significantly less potent *in vivo*, at least as a monotherapy, because of limited bioavailability. Experiments are now under way with a more bioavailable formulation of this drug which appears to have an enhanced effect on viral load. However, for the present, in terms of clinical potency, ritonavir or indinavir appear to represent a better efficacy choice than saquinavir.

The formulation of ritonavir used in clinical trials, a syrup, was difficult to take because of a very bad taste and nausea. This resulted in a considerable minority of patients finding it difficult to comply with continuing medication. The new oral formulation of ritonavir appears to be more tolerable but still produces considerable problems with compliance. It is possible that this may be improved by starting with a lower dose of ritonavir and dose-escalating over 1–2 weeks. At full dose of ritonavir, the initial plasma levels of drug will be very high, falling over the first few weeks of therapy as the cytochrome P450 system becomes induced. Thus, a dose-escalating regimen would reduce the initial very high plasma level and associated nausea and the drug levels with this regimen may be relatively constant over long periods of time. The other major disadvantage of ritonavir is the wide range of other drugs often used in advanced HIV disease which are contraindicated because of their metabolism by the cytochrome P450 system. Other adverse events with ritonavir include diarrhoea, perioral paraesthesia and disturbance of liver function. Saquinavir and indinavir, in contrast, seem to cause less nausea and the range of drugs which are contraindicated appears to be fewer. The side effects of indinavir are relatively infrequent with asymptomatic indirect hyperbilirubinaemia in around 15% of patients and renal colic in 4% being the commonest events. In terms of tolerability, saquinavir appears to be the preferred treatment at present with the most common adverse event being gastrointestinal disturbance.[23]

The rising drug costs in HIV mean pricing of these compounds may be an influential factor on their use; in the United States, indinavir has achieved the lowest annual cost but remains similarly priced to ZDV.

Another important factor in deciding which proteinase inhibitor to use as initial therapy is cross resistance. It is clear from dose-escalating studies that indinavir, particularly in sub-optimal doses, allows the development of HIV with resistance to a range of other proteinases which include saquinavir and ritonavir.[24] Ritonavir resistance is induced by similar mutations within the proteinase enzyme and is also likely to induce cross resistance to indinavir.[25] Saquinavir resistance in clinical treatment appears to be slower to develop than that seen with indinavir or ritonavir[26,27] possibly due to an influence on viral fitness seen with the saquinavir associated mutations.[28] Resistance to saquinavir is generally associated with maintained *in vitro* sensitivity to both indinavir and ritonavir. These additional factors may be very important in deciding the optimum sequence of proteinase inhibitors,[29] particularly if the new formulation of saquinavir with enhanced bioavailability (and presumably enhanced activity) becomes available soon.

Plasma levels of saquinavir are markedly enhanced in animals and in single dose human experiments by the coadministration of ritonavir which acts by inhibiting the major metabolic pathway of saquinavir (cytochrome P450).[30] Thus, the use of ritonavir and saquinavir together would be attractive for two reasons. Firstly the drug combination would enhance the plasma levels of saquinavir and hence increasing the inhibition of HIV replication. Secondly it may be that the concomitant use of two proteinases which do not have an overlapping resistance patterns, would delay the development of clinical resistance to either compound.

Conclusion

Proteinase inhibitors are clearly potent inhibitors of HIV viral replication and as such represent an important advance in the treatment. Most of the present studies are short term, involving small numbers of patients and the larger clinical studies with outcome data are confined at present to individuals with advanced disease. The optimum role of proteinase inhibitors will await further clinical studies but it is already clear that these drugs should always be used in optimum dose and preferably combined with other antiretroviral agents. Whether they should be used in initial combination therapy or reserved for when therapy is failing is unclear. Similarly, it is not known how to sequence these drugs. Current advice for treating physi-

cians and patients is therefore difficult. In individuals with advanced disease it is clear that the addition of a proteinase will prolong life and delay clinical events and therefore patients should be treated with a proteinase inhibitor, preferably together with a nucleoside analogue to which they have not previously been exposed or to which they are not resistant. When the decision to initiate antiretroviral therapy has been made, it remains unclear as to whether or not a proteinase inhibitor should be given at this stage. Such triple combinations would be logical and would have more chance of reducing plasma viraemia below detectable levels. However, compliance, cost, side effects and limitation of future therapeutic options in these patient groups may be important negative factors, particularly if therapy is going to be continued for a number of years. Many patients, certainly in the United Kingdom, will opt for beginning relatively early with two dideoxynucleoside analogues, retaining proteinase inhibitor therapy for later in disease when the initial dideoxynucleoside combinations have clearly failed. At present indinavir appears to be the proteinase inhibiter of choice in terms of efficacy. However, if the new enhanced formulation of saquinavir shows similar activity, the relative lack of side effects and the fact that viruses which become resistant to this drug mostly remain sensitive to both indinavir and ritonavir, might well indicate that this drug should be used as the initial proteinase inhibiter, reserving the other two drugs for later in the course of disease.

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1 Gazzard B. Further results from European/Australian Delta trial. 3rd National Conference on Retroviruses and Opportunistic infections, Washington, 1996: Abstract LB 5.
2 Saravolatz L, Collins G, Hodges D, Winslow D, and the Terry Beirn Community Programs for clinical research on AIDS 007 Protocol team. A randomized comparative trial of ZDV versus ZDV plus ddI versus ZDV plus ddC in persons with CD4 cell counts of < 200/mm³. 3rd National Conference on Retroviruses and Opportunistic infections, Washington, 1996: Abstract LB 4.
3 Hammer S, Katzenstein D, Hughes M, Gundacker M, Hirsch M, Merigan T for the ACTG 175 study team. Nucleoside monotherapy (MT) vs. Combination therapy (CT) in HIV infected adults: a randomized, double-blind placebo controlled trial in persons with CD4 cell counts 200–500/mm³. 35th ICAAC, San Francisco 1995; abstract LB 1.
4 Cameron B, Heath-Chiozzi M, Kravick S, Mills R, Potthoff A, Henry D, *et al.* Prolongation of life and prevention of AIDS in advanced HIV immunodeficiency with ritonavir. 3rd Conference on Retroviruses and Opportunistic infections, Washington Jan 28-Feb 1, 1996; abstract LB6a.
5 Salgo MP, Beattie D, Bragman K, Donatocci L, Jones M, Montgomery L. Saquinavir (Invirase, SQV) vs. HIVID (Zalcitabine, ddC) vs. combination as treatment for advanced HIV infection in patients discontinuing/unable to take retrovir (zidovudine, ZDV). XIth International conference on AIDS, Vancouver, July 1996: abstr Mo.B.410.
6 Kohl NE, Emini EA, Schlief WA, Davis LJ, Heimbach JC, Dixon RA, *et al.* Active human immunodeficiency virus protease is required for viral infectivity. *Proc Natl Acad Sci USA* 1988;**85**:4686–90.
7 Roberts NA, Martin JA, Kinchington D, Broadhurst AV, Craig JC, Duncan IB, *et al.* Rational design of peptide-based HIV proteinase inhibitors. *Science* 1990;**248**:358–61.
8 Craig JC, Duncan IB, Hockley D, Grief C, Roberts NA, Mills JS. Antiviral properties of R031–8959, an inhibitor of human immunodeficiency virus (HIV) proteinase. *Antiviral Res* 1991;**16**:295–305.
9 Johnson VA, Merrill DP, Chou T-C, Hirsch MS. Human immunodeficiency virus type-1 (HIV-1) inhibitory interactions between protease inhibitor Ro31–8959 and zidovudine, 2′, 3′-dideoxycytidine or recombinant interferon-alpha-A against zidovudine-sensitive and -resistant HIV-1 in vitro. *J Infect Dis* 1992;**166**:1143–6.
10 Massari F, Staszewski S, Berry P, Kahn J, Frank I, Heath-Chiozzi M, *et al.* A double-blind, randomized trial of Indinavir (MK-639) alone or with zidovudine vs. zidovudine alone in zidovudine naive patients. 35th ICAAC, San Francisco, September 1995; abstr LB-6.
11 Markowitz M, Saag M, Powderly WG, Hurley AM, Hsu A, Valdes JM, *et al.* A preliminary study of ritonavir, an inhibitor of HIV-I protease, to treat HIV-I infection. *N Engl J Med* 1995;**333**:1534–9.
12 Vella S. Update on a proteinase inhibitor. *AIDS* 1994;**89(Suppl 3)**: S25-S29.
13 O'Brein WA, Harigan PM, Martin D, Esinhart J, Hill A, Benoit S, *et al.* Changes in plasma HIV-1 RNA and CD4 + lymphocyte counts and the risk of progression to AIDS. *N Engl J Med* 1996;**334**:426–31.
14 Mellors J, Steigbigel R, Gulick R, Frank I, Berry P, McMahon D, *et al.* Antiretroviral activity of the oral protease inhibitor, MK-639, in p24-antigenaemic, HIV-1 infected patients with < 500 CD4/mm³. 35th ICAAC, San Francisco, September 1995;abstr I172.
15 Gulick R, Mellors J, Havlir D, Eron J, Gonzalez C, McMahon E, *et al.* Potent and sustained antiretroviral activity of indinavir (IDV) in combination with zidovudine (ZDV) and lamivudine (3TC). 3rd Conference on Retroviruses and Opportunistic infections, Washington Jan 28-Feb 1, 1996; abstract LB7.
16 Massari F, Conant M, Mellors J, Steigbigel R, Milduan D, Greenberg R, *et al.* A phase II open-label, randomized study of the triple combination of indinavir, zidovudine (ZDV) and didanosine (ddI) versus indinavir alone versus zidovudine? didanosine in antiretroviral naive patients. 3rd Conference on Retroviruses and Opportunistic infections, Washington Jan 28-Feb 1, 1996; abstract 200.
17 Mathez D, De Truchis P, Gorin I, Katlama C, Pialovx G, Saimot AG *et al.* Ritonavir, AZT, ddC as a triple combination in AIDS patients. 3rd Conference on Retroviruses and Opportunistic infections, Washington Jan 28-Feb 1, 1996; abstract 285.
18 Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, *et al.* Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *N Engl J Med* 1996; **334**:1011–7.
19 Fauci AS, Pantaleo G, Stanley S, Weissman D. Immunopathogenic mechanisms of HIV infection. *Ann Intern Med* 1996;**124**:654–63.
20 Kellagher AD, Carr A, Zaunders J, Cooper DA. Alterations in the immune response of human immunodeficiency virus (HIV)-infected subjects treated with an HIV-specific protease inhibitor, ritonavir. *J Infect Dis* 1996;**173**:321–9.
21 Fischl MA, Richman DD, Greico MH, Gottlieb MS, Volberding PA, Laskin OL, *et al.* The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind placebo-controlled trial. *N Engl J Med* 1987;**317**:185–91.
22 Saag MS, Holodniy M, Kuritzkes D, O'Brien WA, Coombs R, Poscher ME, *et al.* HIV viral load markers in clinical practice. *Nature Medicine* 1996;**2**:625–9.
23 Moyle G, Gazzard B. Current knowledge and future prospects for the use of HIV protease inhibitors. *Drugs* 1996;**51**:701–12.
24 Condra JH, Schlief WA, Blahy OM, Gabryelski LJ, Graham DJ, Quintero JC, *et al. In vivo* emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature* 1995;**374**:569–71.
25 Molla A, Korneyeva M, Gao Q, Vasavanonda S, Schipper PJ, Mo H-M, *et al.* Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. *Nature Medicine* 1996;**2**:760–6.
26 Vella S, Butto S, Franco M, Olivetta E, Iomino C, Galluzzo C, *et al.* Viral load and CD4 + responses during combination therapy with zidovudine and saquinavir: correlation with emergence of viral isolates with reduced sensitivity. 4th International Workshop on HIV-drug resistance, Sardinia, July 1995; abstr 43.
27 Jacobson H, Yasargil K, Winslow DL, Craiy JC, Krohn A, Duncan IB, Mous J. Characterisation of human immunodeficiency virus type 1 mutants with decreased sensitivity to proteinase inhibitor Ro 31–8959. *Virology* 1995;**206**:527–34.
28 Furfine E, Maschera B, Blair E. Human immunodeficiency virus resistance to saquinavir is mediated by the protease:reduced affinity of mutant proteases for saquinavir occurs from increased dissociation constants. 5th International Workshop on HIV-drug resistance, Whistler July 3–6 1996; abstract 13.
29 Moyle GJ. Use of viral resistance patterns to antiretroviral drugs in optimising selection of drug combinations and sequences. *Drugs* 1996;**52** (in press).
30 Norbeck D, Kumar G, Marsh K, Rodrigues D, McDonald E, Molla A, *et al.* Ritonavir and saquinavir: potential for two-dimensional synergy between HIV protease inhibitors. 35th ICAAC, San Francisco, September 1995; abstr LB7.